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(FILE 'HOME' ENTERED AT 14:53:45 ON 04 OCT 2002)

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CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DRUGB, DRUGLAUNCH, DRUGMONOG2, ...' ENTERED AT 14:53:52 ON 04 OCT 2002

SEA FRUCTOSYLTRANSFERASE OR LEVANSUCRASE

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L1 QUE FRUCTOSYLTRANSFERASE OR LEVANSUCRASE

FILE 'CAPLUS, BIOSIS, PASCAL, BIOTECHNO, SCISEARCH, MEDLINE' ENTERED AT 14:55:54 ON 04 OCT 2002

L2 621 S L1 (P) (INULIN OR LEVAN OR FRUC?-OLIGOSACCHARID OR FRUC?-POLY

L3 21 S L2 AND LACTOBACILLUS

L4 8 DUP REM L3 (13 DUPLICATES REMOVED)

L5 107 S L2 AND (PROCESS OR METHOD OR MAKING)

L6 71 DUP REM L5 (36 DUPLICATES REMOVED)

=> d l4 ibib ab 1-8

L4 ANSWER 1 OF 8 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:696558 CAPLUS
TITLE: Novel fructosyltransferases and their use in recombinant probiotic **lactobacilli**
INVENTOR(S): Van Hijum, Sacha Adrianus Fokke Taco; Van Geel-Schutten, Gerritdina Hendrika; Dijkhuizen, Lubbert; Rahaoui, Hakim
PATENT ASSIGNEE(S): Neth.
SOURCE: U.S. Pat. Appl. Publ., 38 pp., Cont.-in-part of U. S. Ser. No. 604,958.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2002127681	A1	20020912	US 2001-995587	20011129

PRIORITY APPLN. INFO.: EP 2000-201872 A 20000525
US 2000-604958 A2 20000628

AB The present invention describes two novel proteins having **fructosyltransferase** activity. Both enzymes are derived from **lactobacilli**, which are food-grade micro-organisms with the Generally Recognized As Safe (GRAS) status. One of these proteins produces an **inulin** and fructo-oligosaccharides, while the other produces a **levan** and fructo-oligosaccharides. According to the invention **lactobacilli** capable of producing an **inulin** and/or a **levan** and/or fructo-oligosaccharides using one or both of the **fructosyltransferases** can be used as a probiotic or a symbiotic.

L4 ANSWER 2 OF 8 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 1
ACCESSION NUMBER: 2002:684000 CAPLUS
TITLE: Characterization of a novel **fructosyltransferase** from **Lactobacillus** reuteri that synthesizes high-molecular-weight **inulin** and **inulin** oligosaccharides
AUTHOR(S): van Hijum, S. A. F. T.; van Geel-Schutten, G. H.; Rahaoui, H.; van der Maarel, M. J. E. C.; Dijkhuizen, L.
CORPORATE SOURCE: Centre for Carbohydrate Bioengineering, TNO-RUG, University of Groningen, Haren, 9750 AA, Neth.
SOURCE: Applied and Environmental Microbiology (2002), 68(9), 4390-4398
CODEN: AEMIDF; ISSN: 0099-2240
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **Fructosyltransferase** (FTF) enzymes produce fructose polymers (fructans) from sucrose. Here, we report the isolation and characterization of an FTF-encoding gene from **Lactobacillus** reuteri strain 121. A C-terminally truncated version of the ftf gene was successfully expressed in Escherichia coli. When incubated with sucrose, the purified recombinant FTF enzyme produced large amts. of

fructo-oligosaccharides (FOS) with .beta.-(2.fwdarw.1)-linked fructosyl units, plus a high mol.-wt. fructan polymer (>107) with .beta.-(2.fwdarw. linkages (an **inulin**). FOS, but not **inulin**, was found in supernatants of L. reuteri strain 121 cultures grown on medium contg. sucrose. Bacterial **inulin** prodn. has been reported for only Streptococcus mutans strains. FOS prodn. has been reported for a few bacterial strains. This paper reports the first-time isolation and mol. characterization of (i) a **Lactobacillus** ftf gene, (ii) an inulosucrase assocd. with a generally regarded as safe bacterium, (iii) an FTF enzyme synthesizing both a high mol. wt. **inulin** and FOS, and (iv) an FTF protein contg. a cell wall-anchoring LPXTG motif. The biol. relevance and potential health benefits of an inulosucrase assocd. with an L. reuteri strain remain to be established.

REFERENCE COUNT: 48 THERE ARE 48 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 3 OF 8 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2001:868644 CAPLUS
DOCUMENT NUMBER: 136:17259
TITLE: Purification, characterization and use of inulosucrase and levansucrase from **Lactobacillus** reuteri
INVENTOR(S): Van Geel-Schutten, Gerritdina Hendrika; Rahaoui, Hakim; Dijkhuizen, Lubbert; Van Hijum, Sacha Adrianus Fokke Taco
PATENT ASSIGNEE(S): Nederlandse Organisatie Voor Toegepast-Wetenschappelijk Onderzoek, Neth.
SOURCE: PCT Int. Appl., 54 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001090319	A2	20011129	WO 2001-NL392	20010523

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: EP 2000-201872 A 20000525
EP 2001-200049 A 20010109

AB The present invention describes two novel proteins having **fructosyltransferase** activity. One of the enzymes is an inulosucrase which produces an **inulin** and fructo-oligosaccharides, while the other is a **levansucrase** which produces a **levan**. Both enzymes are derived from **Lactobacillus** reuteri, which are food-grade microorganisms with the Generally Recognized As Safe (GRAS) status. Isolation of DNA from L. reuteri, nucleotide sequence anal. of the inulosucrase (ftfA) gene, construction of plasmids for expression of the inulosucrase gene in E. coli Top10, expression of the inulosucrase gene in E. coli Top10 and identification of the polysaccharides produced by the recombinant enzyme are described. Purifn. and amino acid sequencing of the L. reuteri **levansucrase** (gene ftfB) and nucleotide sequence of the gene ftfB are reported. According to the invention **lactobacilli** capable of producing an **inulin** and/or a **levan** and/or

fructo-oligosaccharides using one or both of the **fructosyltransfer[redacted]s** can be used as a probiotic o[redacted] symbiotic.

L4 ANSWER 4 OF 8 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 2
ACCESSION NUMBER: 2001:922622 CAPLUS
DOCUMENT NUMBER: 136:196024
TITLE: Purification of a novel **fructosyltransferase** from **Lactobacillus** reuteri strain 121 and characterization of the **levan** produced
AUTHOR(S): van Hijum, Sacha A. F. T.; Bonting, Kees; van der Maarel, Marc J. E. C.; Dijkhuizen, Lubbert
CORPORATE SOURCE: Microbial Physiology Research Group, Groningen Biomolecular Sciences and Biotechnology Institute (GBB), University of Groningen, Groningen, 9750 AA, Neth.
SOURCE: FEMS Microbiology Letters (2001), 205(2), 323-328
CODEN: FMLED7; ISSN: 0378-1097
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **Fructosyltransferase** (FTF) enzymes have been characterized from various Gram-pos. bacteria, but not from **Lactobacillus** sp. In a screening of 182 **lactobacilli** for polysaccharide prodn. only one strain, **Lactobacillus** reuteri strain 121, was found to produce a fructan being a **levan**. Here we report the first-time identification and biochem. characterization of a **Lactobacillus** FTF enzyme. When incubated with sucrose the enzyme produced a **levan** that is identical to that produced by Lb. reuteri strain 121 cells.
REFERENCE COUNT: 23 THERE ARE 23 CITED REFERENCES AVAILABLE FOR THIS
FORMAT RECORD. ALL CITATIONS AVAILABLE IN THE RE

L4 ANSWER 5 OF 8 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 3
ACCESSION NUMBER: 2000:807451 CAPLUS
DOCUMENT NUMBER: 134:130286
TITLE: Exopolysaccharide production by **Lactobacillus** reuteri, involving sucrase type of enzymes
AUTHOR(S): van Geel-Schutten, G. H.; van Hijum, S. A. F. T.; Kralj, S.; Rahaoui, H.; Leer, R. J.; Dijkhuizen, L.
CORPORATE SOURCE: TNO Voeding, Zeist, 3700 AJ, Neth.
SOURCE: Mededelingen - Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen (Universiteit Gent) (2000), 65(3a), 197-201
CODEN: MFLBER; ISSN: 1373-7503
PUBLISHER: Universiteit Gent, Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review with 5 refs. Exopolysaccharides (EPSs) find numerous applications in the food as well as in the nonfood industries. They can be used as for instance as viscosifying, thickening, gelling or water binding agents. Furthermore certain EPSs are known to exert health promoting effects such as cholesterol lowering, immunomodulating, antitumoral and prebiotic activities. Using a new method, a large collection of **Lactobacillus** strains was screened on the prodn. of EPS. One of the pos. strains, strain 121, produced two different sol. homopolysaccharides during growth on sucrose, a fructan and a glucan. This strain was identified as **Lactobacillus** reuteri, a probiotic strain and an excellent colonizer of the gastrointestinal tract of a broad variety of hosts, including humans. L. reuteri 121 was selected for further research. Structure anal. of the polysaccharides produced by L. reuteri 121 revealed that the fructan was a linear **levan** with

.beta.(2-6)-linked fructosyl units. This was the first example of fructan synthesis by **lactobacilli**. The glucan possessed a unique highly branched structure with .alpha.(1-4) and .alpha.(1-6) linkages with .alpha.(1-4,6) branching points. Both polymers were synthesized by sucrase-type of enzymes (glucosyl- and **fructosyltransferases**). These enzymes only need sucrose as substrate; the energy released by the cleavage of the glycosidic bond in sucrose is subsequently used for the polysaccharide synthesis reaction. During growth of L. reuteri on sucrose or maltose, the sucrases responsible for the synthesis of the glucan and the **levan** appeared to be completely bound to the cell wall, whereas during growth on sucrose part of the enzymes was released into the culture medium. EPS prodn. was not a stable characteristic in continuous cultures. Different spontaneous mutants appeared, such as the EPS-neg. mutant strain K24 which lacks both the glucansucrase (a glucosyltransferase) and the **levansucrase** (a **fructosyltransferase**). Mutant 35-5, lacking **levansucrase**, appeared after a pH shift-down. Using PCR techniques with degenerated primers based on known glucansucrase or **fructosyltransferase** amino acid sequences, chromosomal fragments contg. glucansucrase (gtfA) or **fructosyltransferase** (ftfA) were amplified. Both fragments were sequenced and characterized at the amino acid level and phylogenetic trees of both types of sucrases were constructed. Both the gtfA and the ftfA were cloned sep. in Escherichia coli. Cell free exts. of the E. coli strain harboring the ftfA gene produced an inulosucrase, which synthesized **inulin** and fructose-oligosaccharides from sucrose. The recombinant glucansucrase and the L. reuteri glucansucrase synthesized the same unique glucan. These were the first examples of the isolation, characterization, and cloning of **Lactobacillus** glucansucrase and **fructosyltransferase** genes.

REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 6 OF 8 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 4

ACCESSION NUMBER: 1999:433566 CAPLUS
DOCUMENT NUMBER: 131:196768
TITLE: Biochemical and structural characterization of the glucan and fructan exopolysaccharides synthesized by the **Lactobacillus** reuteri wild-type strain and by mutant strains
AUTHOR(S): Van Geel-Schutten, G. H.; Faber, E. J.; Smit, E.; Bonting, K.; Smith, M. R.; ten Brink, B.; Kamerling, J. P.; Vliegenthart, J. F. G.; Dijkhuizen, L.
CORPORATE SOURCE: Department of Microbiology, TNO Nutrition and Food Research, Zeist, 3700 AJ, Neth.
SOURCE: Applied and Environmental Microbiology (1999), 65(7), 3008-3014
CODEN: AEMIDF; ISSN: 0099-2240
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **Lactobacillus** reuteri LB 121 cells growing on sucrose synthesize large amts. of a glucan (D-glucose) and a fructan (D-fructose) with mol. masses of 3500 and 150 kDa, resp. Methylation studies and 13C or 1H NMR anal. showed that the glucan has a unique structure consisting of terminal, 4-substituted, 6-substituted, and 4,6-disubstituted .alpha.-glucose in a molar ratio of 1.1:2.7:1.5:1.0. The fructan was identified as a (2.fwdarw.6)-.beta.-D-fructofuranan or **levan**, the first example of **levan** synthesis by a **Lactobacillus**

L5 ANSWER 8 OF 8 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 1999274169 EMBASE
TITLE: Purification and immobilization of fructosyl transferase for **production** of **fructo-oligosaccharide**(s) from sucrose.
AUTHOR: Patil V.B.; Patil N.B.
CORPORATE SOURCE: N.B. Patil, Department of Biochemistry, Shivaji University,
Kolhapur 416 004, India
SOURCE: Indian Journal of Experimental Biology, (1999) 37/8 (830-834).
Refs: 16
ISSN: 0019-5189 CODEN: IJEBA6
COUNTRY: India
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB A protocol for commercial **production** of a non digestible sweetner, **fructo-oligosaccharide**(s) from sucrose has been developed. The extracellular enzyme fructosyl transferase was isolated aged purified from Aureobasidium pullulans. The enzyme was covalently immobilized on CNBr activated agarose for its economical viability and for continuous use.

species. Strain LB 121 possesses glucansucrase and **levansucrase** enzymes that occur in a cell-assocd. and a cell-fr state after growth on sucrose, raffinose, or maltose but remain cell assocd. during growth on glucose. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of sucrose culture supernatants, followed by staining of gels for polysaccharide synthesizing activity with sucrose as a substrate, revealed the presence of a single glucansucrase protein of 146 kDa. Growth of strain LB 121 in chemostat cultures resulted in rapid accumulation of spontaneous exopolysaccharide-neg. mutants that had lost both glucansucrase and **levansucrase** (e.g., strain K-24). Mutants lacking all **levansucrase** activity specifically emerged following a pH shiftdown (e.g., strain 35-5). Strain 35-5 still possessed glucansucrase and synthesized wild-type glucan.

REFERENCE COUNT: 50 THERE ARE 50 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 7 OF 8 BIOTECHNO COPYRIGHT 2002 Elsevier Science B.V.

ACCESSION NUMBER: 1999:29065473 BIOTECHNO

TITLE: In vitro digestibility and fermentability of **levan** and its hypocholesterolemic effects in rats

AUTHOR: Yamamoto Y.; Takahashi Y.; Kawano M.; Iizuka M.; Matsumoto T.; Saeki S.; Yamaguchi H.

CORPORATE SOURCE: Dr. H. Yamaguchi, Division Environmental Food Sci., Department Food and Nutrition, Osaka City University, Sugimoto 3-3-138, Sumiyoshi, Osaka 558-8585, Japan.

SOURCE: Journal of Nutritional Biochemistry, (1999), 10/1 (13-18), 35 reference(s)
CODEN: JNBIEL ISSN: 0955-2863

PUBLISHER ITEM IDENT.: S0955286398000771

DOCUMENT TYPE: Journal; Article

COUNTRY: United States

LANGUAGE: English

SUMMARY LANGUAGE: English

AB This study describes the in vitro digestibility and fermentability of high molecular weight (ca. 2,000,000) **levan** and its effect on the metabolism of lipids in growing rats fed cholesterol-free diets. **Levan** was synthesized from sucrose using bacterial **levansucrase** immobilized on a honeycomb-shaped ceramic support. Although body weight gain, weight of visceral organs, morphologic changes in the digestive tract, and the serum triacylglycerol and glucose concentrations were not affected by feeding **levan** diets for 4 weeks, a significant hypocholesterolemic effect was observed. Serum cholesterol level was decreased to 83% or 59% by feeding a 1% or 5% **levan** diet, respectively. The hypocholesterolemic effect was accompanied by a significant increase in fecal excretion of sterols and lipids. High molecular weight **levan**, though not hydrolyzed by the salivary amylases, was hydrolyzed by artificial gastric juice and was changed to a low molecular weight (ca. 4,000) **levan** with a small amount of fructose, but did not produce any fructooligosaccharides. Low molecular weight (ca. 6,000) **levan** was not hydrolyzed by either pancreatic juice or small intestinal enzymes. This suggests that, in vivo, low molecular weight **levan** derived from the high molecular weight material is not further digested and reaches the colon intact. The fermentation of low molecular weight **levan** (ca. 6,000) by several strains of bifidobacteria was not observed. These results showed that the hypocholesterolemic effect of **levan** may result from the prevention of intestinal sterol absorption, and not from the action of the fermentation products of **levan**. Copyright (C)

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L4 ANSWER 8 OF 8 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1975:591718 CAPLUS
DOCUMENT NUMBER: 83:191718
TITLE: Variations in microbial and biochemical components of four-day plaque during a four-week controlled diet period
AUTHOR(S): Dennis, D. Adele; Gawronski, Thomas H.; Sudo, Sara Z.; Harris, Robert S.; Folke, Lars E. A.
CORPORATE SOURCE: Sch. Dent., Univ. Minnesota, Minneapolis, Minn., USA
SOURCE: J. Dent. Res. (1975), 54(4), 716-22
CODEN: JDREAF
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Variation in microbial and biochem. components of human 4-day plaque was studied in subjects maintained on a high-sucrose [57-50-1] diet during 4 weeks. Significant changes in populations of **lactobacilli** dextranase-producing organisms, Streptococcus mutans, and S. sanguis occurred during this period. Except for specific amylase activity, all other biochem. parameters (total carbohydrate and buffer-sol. carbohydrate contents, specific activities of glucosyltransferase, **fructosyltransferase**, dextran hydrolase, **levan** hydrolase, and invertase) either remained const. or exhibited insignificant variation during the 4-week diet period. Specific amylase activity was attributed to salivary contamination.

=> d l6 ibib ab 61-71

L6 ANSWER 61 OF 71 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1990:496167 CAPLUS
DOCUMENT NUMBER: 113:96167
TITLE: Production of substantially pure fructose
INVENTOR(S): Hatcher, Herbert J.; Gallian, John J.; Leeper, Stephen A.
PATENT ASSIGNEE(S): Idaho Research Foundation, Inc., USA
SOURCE: U.S., 6 pp.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 4927757	A	19900522	US 1988-225914	19880729

AB Substantially pure fructose is prepd. from a sucrose-contg. substrate. First, sucrose is converted to levan and glucose with a fructosyl transferase (I) and the levan hydrolyzed to fructose with levanase. This **method** may also be used to prep. a high-fructose syrup contg. .apprx.60% fructose. Sucrose-contg. substrate prepd. from crushed sugar beet was incubated with I-producing Microbacterium laevaniformans at 26.degree.-28.degree. and ultrafiltered to obtain levan. Levan was the hyddrolyzed to fructose using a hollow fiber membrane filtercontg. immobilized levanase. The hydrolyzate was then concd. by reverse osmosis or hyperfiltration to obtain a high-fructose syrup contg. 60% fructose.

L6 ANSWER 62 OF 71 BIOTECHNO COPYRIGHT 2002 Elsevier Science B.V.
ACCESSION NUMBER: 1990:21036993 BIOTECHNO
TITLE: Bacillus subtilis **levansucrase**: Amino acid substitutions at one site affect secretion efficiency and refolding kinetics mediated by metals
AUTHOR: Petit-Glatron M.F.; Monteil I.; Benyahia F.; Chambert R.
CORPORATE SOURCE: Lab. Genetique/Membranes, Institut Jacques Monod, CNRS Universite Paris VII, 2 Place Jussieu,75251 Paris, France.
SOURCE: Molecular Microbiology, (1990), 4/12 (2063-2070)
CODEN: MOMIEE ISSN: 0950-382X
DOCUMENT TYPE: Journal; Article
COUNTRY: United Kingdom
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Studies of the equilibrium between native and denatured forms of wild-type **levansucrase** showed that the denatured form was predominant at 37.degree.C and pH 7 in the absence of free metal. The shift to the native form was promoted by metal ions such as Fe.sup.3.sup.+ or Ca.sup.2.sup.+. This metal-dependent refolding **process** was not observed in **levansucrase** variants bearing the amino acid substitution Gly-366.fwdarw.Asp or Gly-366.fwdarw.Val. These variants were only slightly secreted by Bacillus subtilis although their signal sequences were normally cleaved and their exocellular forms stable. In contrast, the Gly-366.fwdarw.Ser

variant was secreted at near-normal levels and shared a part of the in vitro refolding properties of the wild-type protein. These differential properties might be related to the ability of the altered region to form a .beta.-form structure. We discuss the possible role of metal ions in the coupling of protein folding and secretion.

L6 ANSWER 63 OF 71 BIOTECHNO COPYRIGHT 2002 Elsevier Science B.V.
ACCESSION NUMBER: 1990:20041980 BIOTECHNO
TITLE: Secretion of Bacillus subtilis **levansucrase**. Fe(III) could act as a cofactor in an efficient coupling of the folding and translocation **processes**
AUTHOR: Chambert R.; Benyahia F.; Petit-Glatron M.-F.
CORPORATE SOURCE: Institut Jacques Monod, Laboratoire Genetique et Membranes, Centre National de la Recherche Scientifique, Universite Paris 7, 2 place Jussieu, 72521 Paris Cedex 05, France.
SOURCE: Biochemical Journal, (1990), 265/2 (375-382)
CODEN: BIJOAK ISSN: 0264-6021
DOCUMENT TYPE: Journal; Article
COUNTRY: United Kingdom
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The refolding of **levansucrase** denatured by urea was studied as a possible model for the second step of the secretion pathway of this protein. The folding-unfolding transition was monitored by measuring intrinsic fluorescence and resistance to proteolysis. Both **methods** provided the same estimation for the unfolding free energy of **levansucrase**, .DELTA.G(D), which was 30.1 .+-. 1.7 kJ .midldot. mol.sup.-.sup.1) (7.2 .+-. 0.4 kcal .midldot. mol.sup.-.sup.1) at pH 7 in 0.1 M-potassium phosphate buffer. The rate of refolding was greatly enhanced by Fe.sup.3.sup.+, whereas the Fe.sup.3.sup.+ chelator EDTA prevented correct refolding. Fe.sup.3.sup.+ allowed the protein to reach its folded form in medium in which the dielectric constant had been lowered by ethanol. The efficiency in vivo of the export of **levansucrase** bearing an amino acid modification which blocks the second step of the translocation pathway was greatly increased by high concentrations of Fe.sup.3.sup.+ in the culture medium. Assuming that the protein folding governs the second step of the secretion **process** of **levansucrase**, we discuss from an irreversible thermodynamic point of view the possible role of Fe.sup.3.sup.+ in the efficient coupling of the two events.

L6 ANSWER 64 OF 71 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1990:457392 CAPLUS
DOCUMENT NUMBER: 113:57392
TITLE: Levan production with a flocculent strain of Zymomonas mobilis
AUTHOR(S): Reiss, M.; Hartmeier, W.
CORPORATE SOURCE: Inst. Food Technol., Hohenheim Univ., Stuttgart, D-7000/70, Fed. Rep. Ger.
SOURCE: Food Biotechnol. (N. Y.) (1990), 4(1), 69-75
CODEN: FBIOEE; ISSN: 0890-5436
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **Levan** is a fructose polymer with potential importance as fructose source of thickening agent in food technol. in case that it could be produced in a simple and cheap **process**. **Levan** concns. of up to 42.5 g/L and a yield from fructose of 0.32 g/g were obtained with a new flocculent mutant of Z. mobilis. A sucrose concn. of 250 g/L, pH-values around 5.0 and temps. of 27 to 30.degree. were optimal.

Higher pH-values and high starting concns. of **levan** caused hydrolysis of the polymer by the bacterium itself. However, adding **levan** up to 1.5 g/L led to nearly 40% increase of the **levan** synthesis. Sorbitol also increased **levan** formation due to a decrease of sucrose and **levan** hydrolysis. Presumably by induction of the **levansucrase**, fructose addn. caused a higher **levan** yield too. In continuous operation, a **levan** productivity of 16 g/Lh and an ethanol productivity of 26.3 g/L.cntdot.h resulted. That was 5 to 12 times, resp. the productivity of batch **processes**.

L6 ANSWER 65 OF 71 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 12
ACCESSION NUMBER: 1989:191177 CAPLUS
DOCUMENT NUMBER: 110:191177
TITLE: Batch and continuous production of levan with flocculent cells of Zymomonas mobilis
AUTHOR(S): Reiss, M.; Hartmeier, W.
CORPORATE SOURCE: Inst. Lebensmitteltechnol., Univ. Hohenheim, Stuttgart, D-7000/70, Fed. Rep. Ger.
SOURCE: Chem., Mikrobiol., Technol. Lebensm. (1989), 12(1), 1-7
CODEN: CMTLBX; ISSN: 0366-7154
DOCUMENT TYPE: Journal
LANGUAGE: German
AB With a flocculent mutant of Z. mobilis **levan** (I) concns. .ltoreq.42.5 g/L and a yield of 0.32 g/g fructose were achieved. Media with a sucrose concn. of 250 g/L, pH 5.0, and temp. of 27-30.degree. were optimal. At higher pH and high starting concns. of I, much of the product was hydrolyzed by the bacterium itself. In contrast, addns. of .ltoreq.1.5 g I/L led to nearly 40% increase of I prodn. Due to a decrease of sucrose and hydrolysis, prodn. was also increased by sorbitol. Presumably by induction of the **levansucrase**, fructose addn. also resulted in higher yields. In continuous operation, I productivity of 16 g/L-h and EtOH productivity of 26.3 g/L-h resulted, 5 and 12-fold, resp., the productivity of batch **processes**.

L6 ANSWER 66 OF 71 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 13
ACCESSION NUMBER: 1980:71419 CAPLUS
DOCUMENT NUMBER: 92:71419
TITLE: The molecular structure of low and high molecular weight **levans** synthesized by **levansucrase**
AUTHOR(S): Tanaka, Toshio; Oi, Susumu; Yamamoto, Takehiko
CORPORATE SOURCE: Fac. Sci., Osaka City Univ., Osaka, 558, Japan
SOURCE: J. Biochem. (Tokyo) (1980), 87(1), 297-303
CODEN: JOBIAO; ISSN: 0021-924X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **Levan** synthesized by Bacillus subtilis **levansucrase** in the presence of alcs. was only of high mol. wt., whereas in solns. of high ionic strength only low-mol.-wt. **levan** was produced. The addn. of low-mol.-wt. **levan** to the enzyme reaction mixt. at low ionic strength stimulated synthesis of a high-mol.-wt. **levan**, but the **levan** added was not incorporated into this high-mol.-wt. **levan**. Methylation anal. revealed that low-mol.-wt. **levans** contained glucose, which was isolated as 2,3,4,6-tetra-O-methylalditol acetate, showing that the glucose units exist as terminal residues. The mol. wt. of **levan** estd. on the basis of glucose content coincided with that detd. by the gel filtration **method**. Methylation anal. also revealed that the no. of fructose residues of the linear fraction linked by .fwdarw.6(Fru)2.fwdarw. bonds was 22 for **levan** with a mol. wt. of (8.4-22) .times. 103, whereas it was 11 for that of 2000 .times. 103-mol.-wt. **levan**.

The no. of .fwdarw.6(.fwdarw.1)(Fru)2.fwdarw. branched residues increased with the increase the mol. wt. of the **levan** syn sized.

L6 ANSWER 67 OF 71 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 14
ACCESSION NUMBER: 1978:147965 CAPLUS
DOCUMENT NUMBER: 88:147965
TITLE: Levansucrase of Bacillus subtilis
AUTHOR(S): Tanaka, Toshio; Oi, Susumu; Iizuka, Masaru; Yamamoto, Takehiko
CORPORATE SOURCE: Fac. Sci., Osaka City Univ., Osaka, Japan
SOURCE: Agric. Biol. Chem. (1978), 42(2), 323-6
CODEN: ABCHA6; ISSN: 0002-1369
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **Levansucrase** of B. subtilis saccharolyticus was purified from the supernatant of an aerobically incubated suspension of the cells in a dil. sucrose soln. by chromatog. on DEAE-cellulose. **Levan** synthesis by the purified enzyme was optimum at low temps., .apprx.0.degree.. The **levan** synthesized was isolated by passing the enzyme reaction mixt. through a DEAE-cellulose column followed by EtOH pptn. **Levan** was isolated in the pure state ([.alpha.]D20,-47). Its mol. wt. was 2 .times. 104 by the gel filtration **method**.

L6 ANSWER 68 OF 71 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 15
ACCESSION NUMBER: 1972:11764 CAPLUS
DOCUMENT NUMBER: 76:11764
TITLE: Biosynthesis of **levan** and a new **method** for the assay of **levansucrase** activity
AUTHOR(S): Ceska, M.
CORPORATE SOURCE: Dep. Biochem., Pharm. AB, Uppsala, Swed.
SOURCE: Biochem. J. (1971), 125(1), 209-11
CODEN: BIJOAK
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The polysaccharide, **levan**, was synthesized in a solidified agar medium contg. sucrose as a source of fructose. The biosynthesis was achieved by the enzyme, **levansucrase** (EC 2.4.1.10), a small quantity of which was placed in circular wells cut in the agar gel. The enzyme slowly diffused through the agar-sucrose medium and the synthesis of **levan** was obsd. as circular white areas, the size of which was dependent on the time of incubation and the concn. of enzyme used.

L6 ANSWER 69 OF 71 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1964:32900 CAPLUS
DOCUMENT NUMBER: 60:32900
ORIGINAL REFERENCE NO.: 60:5904g-h
TITLE: Identity of the sucrase of Bacillus subtilis Marburg with the levansucrase of B. subtilis var nigra
AUTHOR(S): Jozon-Toulouse, Edith; Dedonder, Raymond
CORPORATE SOURCE: Inst. Pasteur, Paris
SOURCE: Compt. Rend. (1963), 257(5), 1184-7
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable
AB cf. CA 57, 3908d. A strain of B. subtilis var. nigra produces a sucrose .fwdarw. **levan**-.beta.-fructofuranosyl transferase and **levan** accumulates in the medium on incubation of B. subtilis var. nigra with sucrose. Strains of B. subtilis Marburg split sucrose without apparent formation of **levan**. The sucrase produced by B. subtilis was prepd. by sonic disruption of the cells in 0.01M phosphate buffer, removal of the cell debris by centrifugation and pptn. with 70-100% satd. (NH4)2SO4. The nucleic acids were removed with 0.25M MgCl2 and the enzyme purified by chromatography on a column of hydroxylapatite and eluted with a gradiant of 0.1-2.0M phosphate buffer (pH 6.0). The fraction eluted between 0.5 and 0.8M was identical with the enzyme prepd.

from B. subtilis var. nigra; it produced **levan** as the nigra var. Its affinity cons[redacted]is 5.4 .times. 10-2M for sucro[redacted]while 5.0 .+-. 10-2M was found for pur[redacted]ed **levansucrase** from B. subtil[redacted]var nigra. Both enzymes need the addn. of starter **levans** of low mol. wt. Immunological identity of both enzymes was proven with the Ouchterlony **method** and other procedures. The amt. of enzyme produced by B. subtilis is only 7% of thai produced by the nigra var.

L6 ANSWER 70 OF 71 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1956:28559 CAPLUS
DOCUMENT NUMBER: 50:28559
ORIGINAL REFERENCE NO.: 50:5812a-d
TITLE: Synthesis of sucrose and other .beta.-D-fructofuranosyl aldosides by levansucrase
AUTHOR(S): Hestrin, Shlono; Feingold, David S.; Avigad, Gad
CORPORATE SOURCE: Hadassah Med. School, Jerusalem
SOURCE: J. Am. Chem. Soc. (1955), 77, 6710
CODEN: JACSAT; ISSN: 0002-7863
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable
AB cf. C.A. 38, 5524.4. In the **levansucrase** system a **process** is catalyzed in which the aglycone of .beta.-D-fructofuranosyl aldosides is transferred reversibly to the anomeric C position of an aldose. A cell-free soln. of **levansucrase** of Aerobacter levanicum allowed to act on raffinose formed **levan**, fructose, and melibiose, but neither glucose nor galactose. In the presence of added D-glucose, raffinose with **levansucrase** formed little **levan** but there was a rapid formation of sucrose. Neither dextransucrase by itself nor a mixt. of dextransucrase and **levansucrase** formed dextran from raffinose alone, but on the addn. of glucose, dextran was formed rapidly. When **levansucrase** acted on sucrose plus melibiose, raffinose was formed. **Levansucrase** formed .alpha.-D-xylopyranosyl-.beta.-D-fructofuranoside, [.alpha.]D20 62.degree.. The following aldoses were converted to the corresponding aldosyl-.beta.-D-fructofuranosides in the presence of **levansucrase** : D-xylose, L-arabinose, D-glucose, D-galactose, and melibiose.

L6 ANSWER 71 OF 71 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1955:61130 CAPLUS
DOCUMENT NUMBER: 49:61130
ORIGINAL REFERENCE NO.: 49:11772c-f
TITLE: Mechanism of degradation and synthesis and some biological activities of intercellular bacterial polysaccharides
AUTHOR(S): Hestrin, Shlomo
CORPORATE SOURCE: Hebrew Univ.-Hadassah Med. School, Jerusalem
SOURCE: 6th Intern. Congr. Microbiol., Symposium-Microbial Metabolism, Suppl. Rend. ist. super. Sanita (1953) 63-70
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable
AB Aerobacter levanicum, a **levan** former, does not degrade **levan**; Azotobacter chroococcum and Bacillus asterosporus, **levan** formers, do. The **levan**-degrading enzyme formation is an adaptive **process** induced by growth on **levan** and sucrose. The metabolism of sucrose via **levan** by **levan** formers involves a reaction sequence in which 2 irreversible hydrolytic steps succeed the irreversible initial polymerative cleavage of the disaccharide: (1) sucrose .fwdarw.**levansucrase** **levan** + glucose; (2) native **levan** + H2O .fwdarw.levanpolyase limit oligolevans; (3) limit oligolevans + H2O .fwdarw.levanoligase or sucrase fructose. Macromol. **levan** and dextran administered intravenously or intraperitoneally promote infection. **Levan** and dextran degraded to the mol.-wt. level of plasma expanders do not. The theoretical mechanism of synthesis is that sucrose and related oligosaccharides are specifically fitted by the preformed interglucosidic

linkage to serve in metabolism as substrates of polysaccharide synthesis. Nonviable, active ried Acetobacter xylinum cells with O as the electron acceptor, polymerized glucose to cellulose, but when ferricyanide and dichloroindophenol replaced O, cellulose was not synthesized. Dried cells exposed to acetone-ether lost their activity.

L4 ANSWER 159 OF 163 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1964:32900 CAPLUS
DOCUMENT NUMBER: 60:32900
ORIGINAL REFERENCE NO.: 60:5904g-h
TITLE: Identity of the sucrase of Bacillus subtilis Marburg with the levansucrase of B. subtilis var nigra
AUTHOR(S): Jozon-Toulouse, Edith; Dedonder, Raymond
CORPORATE SOURCE: Inst. Pasteur, Paris
SOURCE: Compt. Rend. (1963), 257(5), 1184-7
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable

AB cf. CA 57, 3908d. A strain of B. subtilis var. nigra **produces** a sucrose .fwdarw. **levan**-.beta.-fructofuranosyl transferase and **levan** accumulates in the medium on incubation of B. subtilis var. nigra with sucrose. Strains of B. subtilis Marburg split sucrose without apparent formation of **levan**. The sucrase **produced** by B. subtilis was prepd. by sonic disruption of the cells in 0.01M phosphate buffer, removal of the cell debris by centrifugation and pptn. with 70-100% satd. (NH4)2SO4. The nucleic acids were removed with 0.25M MgCl2 and the enzyme purified by chromatography on a column of hydroxylapatite and eluted with a gradiant of 0.1-2.0M phosphate buffer (pH 6.0). The fraction eluted between 0.5 and 0.8M was identical with the enzyme prepd. from B. subtilis vat. nigra; it **produced levan** as the nigra var. Its affinity const. is 5.4 .times. 10-2M for sucrose while 5.0 .+-. 10-2M was found for purified levansucrase from B. subtilis var nigra. Both enzymes need the addn. of starter **levans** of low mol. wt. Immunological identity of both enzymes was proven with the Ouchterlony **method** and other procedures. The amt. of enzyme **produced** by B. subtilis is only 7% of thai **produced** by the nigra var.

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☐ 1. Document ID: US 20020127681 A1

L3: Entry 1 of 3 File: PGPB Sep 12, 2002

PGPUB-DOCUMENT-NUMBER: 20020127681
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020127681 A1

TITLE: Novel fructosyltransferases

PUBLICATION-DATE: September 12, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Van Hijum, Sacha Adrianus Fokke Taco	Groningen		NL	
Van Geel-Schutten, Gerritdina Hendrika	Driebergen-Rijsendberg		NL	
Dijkhuizen, Lubbert	Zuidlaren		NL	
Rahaoui, Hakim	Amersfoort		NL	

US-CL-CURRENT: 435/193; 435/101, 435/252.3, 435/325, 435/69.1, 536/123, 536/23.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

☐ 2. Document ID: US 3879545 A

L3: Entry 2 of 3 File: USPT Apr 22, 1975

US-PAT-NO: 3879545
DOCUMENT-IDENTIFIER: US 3879545 A

TITLE: Vaccines for the prevention of dental caries

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

☐ 3. Document ID: US 20020127681 A1 WO 200190319 A2 AU 200160791 A

L3: Entry 3 of 3 File: DWPI Sep 12, 2002

DERWENT-ACC-NO: 2002-114287
DERWENT-WEEK: 200262
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TITLE: New enzymes having fructosyltransferase activity (e.g. inulosucrase or levansucrase), useful for producing useful levans, inulins and fructo-oligosaccharides from sucrose, which are particularly useful as prebiotic substrates

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

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